A METHOD TO COLLECT HIGH VOLUMES OF MILK FROM MICE (*MUS MUSCULUS*)

Running title: A method to collect to high volumes of milk from mice

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ABSTRACT

Collecting milk samples from mice (*Mus musculus*) may be interesting for a variety of preclinical research. References in the literature for protocols describing how to milk a dam are scarce, and a major limitation of such protocols is the small sample volume that is generally collected. The aim of our study was to develop a practical protocol to collect substantial amounts of milk from mice. Adult female outbred NMRI and inbred BALB/c mice with nursing litters were used in this study. The milking was carried out on days 7–12 after parturition. The pups were separated from their mothers for 6–12 h before milking to allow accumulation of milk in the glands. Dams were anesthetized using either an injectable mixture of midazolam and ketamine, or by use of the inhalational agent isoflurane. To induce milk flow, the mice were given 2-8 IU of oxytocin intraperitoneally. The milk was collected using an electric human breast pump that was modified to accommodate mouse nipples and to handle small liquid volumes. With this protocol, the total amount of milk collected from each dam per each milking ranged between 0.2 and 1.5 mL. We concluded that this milking method provides an excellent means for acquiring substantial amounts of mouse milk.

Key words: Milk, mouse, milking method, animal model.

RESUMEN

La obtención de leche a partir de ratones de laboratorio (*Mus musculus*) podría ser interesante para una gran variedad de estudios preclínicos. Son muy escasas las referencias en la literatura científica sobre protocolos que describan como obtener estas muestras, siendo la principal limitación de tales protocolos el pequeño volumen de muestra que es habitual obtener. El objetivo de nuestro estudio fue desarrollar un método funcional para obtener fácilmente cantidades considerables de leche de ratón. Hembras adultas de la variedad no consanguíneas NMRI y de la consanguínea BALB/c con su camada fueron utilizadas en este estudio. La leche fue recogida entre los días 7-12 tras el parto. Las crías fueron separadas de sus madres entre 6-12 horas antes del ordeño para permitir la acumulación de leche en las glándulas. Las hembras fueron anestesiadas usando o una mezcla de midazolam y ketamina, o empleando isoflurano como agente inhalatorio. Para inducir la eyección de la leche, se administro una dosis de 2-8 UI de oxitocina intraperitonealmente. La leche fue recogida usando un sacaleches eléctrico para humanos que fue modificado para adaptarse al pezón de los ratones y para recoger pequeños volúmenes. Con este procedimiento, la cantidad de leche obtenida de los ratones osciló entre los 0,2 y los 1,5 ml. Concluimos por tanto, que este método proporciona una excelente manera de adquirir cantidades considerables de leche de ratón.

Palabras clave: Leche; ratón; método de ordeño; modelo animal.

INTRODUCTION

Collection of milk from mice (*Mus musculus*) may be a critical part for a variety of preclinical studies such as mammary gland biology, lactation, infant nutrition, and toxicological evaluation of novel drugs (Jolois et al., 2001; Case and Domant, 2012; Demon et al., 2012). Protocols describing how to collect milk from a mouse are scarce in scientific literature. Common features of such protocols are the manually generated vacuum and the small sample volume that is normally collected (De Peters and Hovey, 2009). The aim of our study was to develop a milking protocol for mice that is practical and produces a higher volume of milk.

MATERIALS AND METHODS

Mice, housing and husbandry

A total of 20 female adult mice—7 outbred HsdWinin:NMRI (bred and supplied by a breeding colony of the Central Animal Laboratory at University of Turku, Finland); 3 outbred Sca:NMRI mice (bred and obtained from Scanbur Ltd., Sollentuna, Sweden), and 10 inbred BALB/cOlaHsd mice (supplied by Harlan Laboratories®, Horst, Netherlands and bred in a facility of the Central Animal Laboratory, University of Turku, Finland)—were used in this study. The mice were selected based on availability of dams with a nursing litter from the breeding colony. At the commencement of the milking process, the dams ranged in body weight between 28 and 47 g. All the mice were maintained and milked in a facility of the Central Animal Laboratory, University of Turku, Finland. The mice were housed in top-filtered stainless steel cages (365 x 207 x140 cm) with solid bottoms and Aspen chips as bedding (Tapvei Ltd, Kaavi, Finland), with substantial nesting material. Cages were changed twice a week. The environment in the mouse room consisted of a temperature range of 22 to 27 °C, a relative humidity of 50 to 60%, and artificial illumination with a 12-h light/dark cycle (lights on at 06:00 am). Throughout the study period, all the mice were fed a standard mouse chow (SDS, Special Diet Services, Witham, Essex, UK) *ad libitum*, and tap water was provided without restrictions in polycarbonate bottles. Prior to the milking, all the dams were determined to be healthy based on clinical observations, and were considered pathogen-free based on the results of routine microbiological screen-

ing performed in the colony in accordance with current European recommendations (Nicklas et al., 2002). This study was part of the pilot experiments performed to optimise handling and treatment of another study approved by the National Ethics Committee for Animal Experiments in Finland (ESLH-2009-04845/Ym-23).

Construction of the milking machine

An electric off-the-shelf human breast pump was acquired from a local infant supply store. The original tubing of the milking machine was partially replaced with smaller gauge tubes. The flap valve unit from the machine was modified to function without the original collection vessel. The original breast cup was fitted with diameter reducers and connected to a new collection vessel. All connections and contacting surfaces were sealed using Pechiney Plastics Parafilm M* laboratory wrapping film to avoid pressure loss due to leakage (Figures 1 and 2).

A new collection vessel fit for small liquid volumes was constructed from a small empty glass bottle, a rubber cap for the vial, a 2.0 mL collection tube and two 18G hypodermic needles. All parts were sterilized using 70% ethanol. Pre-sterilized parts in autoclave were used when possible.

A small hole punctured the rubber cap. The tube connected to the milking machine was inserted through the hole. A hypodermic needle, without a plastic syringe attachment, was inserted through the vial cap. The tube was attached to this needle. Another needle was inserted into the free end of the tube to provide a suction cup for a nipple.

The milk collection tube was placed inside the bottle, needle piercing the bottle cap was aimed at the collection tube and the cap was closed. The cap was sealed using Pechiney Plastics Parafilm M* laboratory wrapping film (Figure 3).

The milking machine was turned on and all seals were checked for possible pressure leaks by listening for an audible hiss. Suction pressure was checked by placing the suction cup against a hand covered in a laboratory glove, to which the cup should stick if proper suction was present.

Figure 1. **Modified electric breast pump and a glass vial for milk collection.**

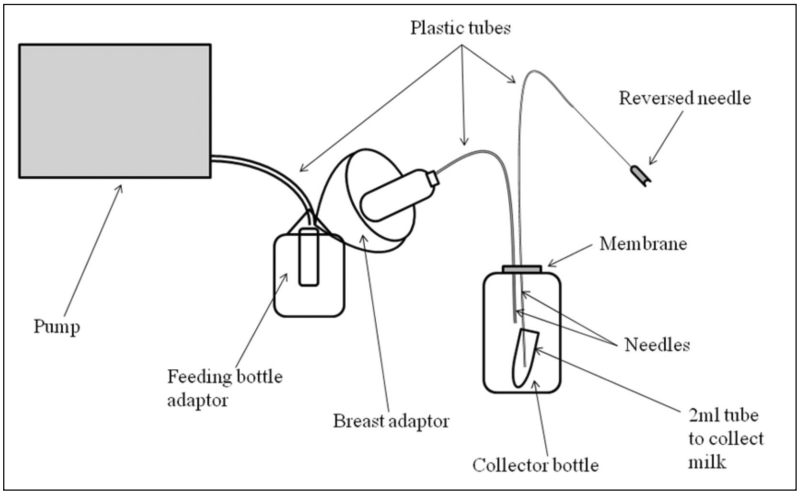

Figure 2. **Milking machine diagram. The different parts are out of scale.**

Figure 3. **A glass vial containing a collection tube. The tube with larger diameter (left) is connected to the milking machine, and the tube with smaller diameter (right) is connected to an inverted needle serving as a suction cup.**

Milking protocol

Milking was carried out during the days of higher milk production, which is between days 7-12 after the parturition. The pups were separated from their mothers for 6-12 h before milking to allow the milk to accumulate in the mammary glands.

Oxytocin was prepared by pipetting 0.8-0.9 ml oxytocin (Partoxin® Vet. 17 µg/ml, 10 U.I./ml) into 1.5 ml Eppendorf tubes. To avoid acidosis of the animal, the pH of the oxytocin was neutralized by adding bicarbonate buffer to the oxytocin until pH is approximately 7. The amount of bicarbonate buffer needed depends on molarity of the buffer and the acid used in the commercial oxytocin solution. The pH was measured with specific strips and adjusted before milking.

A mixture of midazolam (Dormicum® 5 mg/ml) and ketamine (Ketalar® 10 mg/ml) to produce a solution containing 5 mg midazolam/kg bodyweight and 75 mg ketamine/kg bodyweight was prepared to be used as injectable anaesthesia of the milking dam. The animal to be milked was weighed and the amount of solution needed for anaesthesia then calculated.

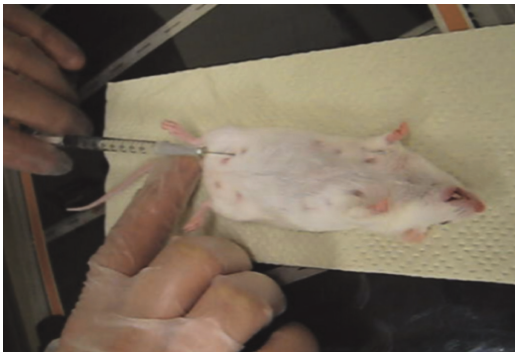

Figure 4. **IP administration of oxytocin between inguinal nipples.**

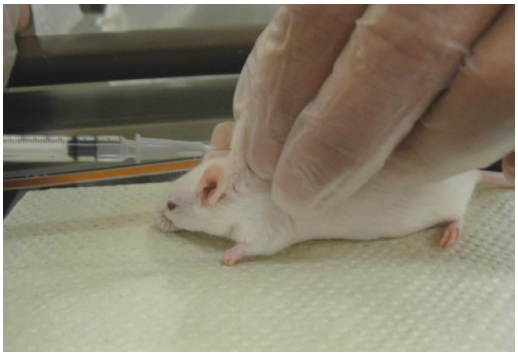

Figure 5. **Rehydration of the dam after milking.**

The anaesthetic solution was injected subcutaneously. After the injection, the animal was placed back in its cage until the effects of anaesthesia were observed. When the animal was unconscious, it was placed on the heating mat to maintain the animal's warmth during the milking process. The depth of anaesthesia was confirmed by checking for lack of pedal reflex.

If the animal started to regain consciousness during the milking an injectable anaesthetic, approximately 1/3 of the initial amount of the anaesthetic, was injected intraperitoneally to the right side of the animal. When the animal is held on its back with its abdomen facing up, its right side will be on the left. This was done to avoid damage to the cecum of the animal.

When using injectable anaesthetics, animals were placed in their respective cages after the milking procedure, and the cage was left on a heating pad until the animal showed signs of recovery (i.e. was aware of its surroundings and started moving around).

Alternatively, inhalational agent isoflurane (Isoflurane®*)* was used for anaesthesia. For induction, a concentration of 4-5% was used, and for maintenance, the concentration of isoflurane was lowered to approximately 2-3%.

After an animal was anesthetized, 2-8 IU of oxytocin was administered intraperitoneally to induce milk flow. Half of the volume was injected between the right inguinal nipples and the other half was injected between the left inguinal nipples (Figure 4).

After milking, the animal was rehydrated using 0.5 ml of a solution consisting of 0.40 ml glucose solution (50 mg/ml) and 0.1 ml physiological saline (9 mg/ml) (Figure 5).

RESULTS AND DISCUSSION

Collection of milk (video)

Milk started flowing within 4-10 min after the oxytocin injection. Then the milking machine was turned on and the amount of suction was adjusted. The suction head of the milking machine was placed on one of the inguinal nipples (Figure 6). Four nipples were used for milking. When the amount of milk received decreases, another nipple was milked. Nipples that have been already milked can be returned to later on.

When no additional milk was produced from the nipples, the milking machine was stopped and the milk stored.

As isoflurane anaesthesia exerted worse results in volume of milk collected and mouse recovery compared with injectable anaesthesia, this method was obviated. The negative effects could be due to the reported inhibitory effect

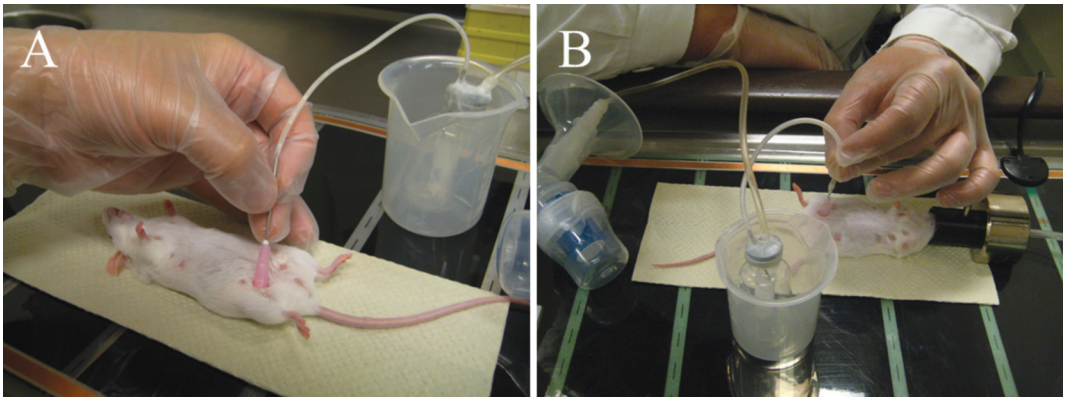

Figure 6. **Milking a mouse using A) injectable (midazolam-ketamine); or B) inhalational anesthesia.**

Figure 7. **Milk collected from a HsdWinin: NMRI dam.**

of the inhalational hydrocarbonated ethers in contractions induced with oxytocin (Yildiz *et al.,* 2005; Gultekin *et al.,* 2006) and the reduction of prolactin levels in blood (Chassagne *et al.,* 2000).

The milk (Figure 7) can be stored up to 3 h in 4ºC, and 5 months in -20ºC and 8 months in -80ºC, with the aim to preserve initial levels of vitamins and long chain polyunsaturated fatty acids (Romeu-Nadal *et al.,* 2008).

For outbred NMRI mice the volume range of milk collected was between 0.5-1.5 ml, and for inbred BALB/cOlaHsd mice the volume collected was between 0.2-0.4 ml. This difference may be in relation with the size of the animal, with a weight between 25-40 g and 15-20 g respectively, or with the genetic background of these two different mouse strains.

About possible anatomical, physiological or histological abnormalities in the mammary glands such as mastitis, we recommend further studies to check the sensibility of the specific strain to the milk collection. If abnormalities in the mammary gland of the mice are suspected, it is recommended to treat the animals in accordance with good veterinary practice.

CONCLUSIONS

In the present methodology, an effective milking method to extract high volumes of milk from mice is described. With this protocol, the total amount of milk collected from dams yielding milk in each milking ranged between 0.2 and 1.5 ml. In particular, maximal milk production was achieved from outbred and multiparous dams with higher bodyweight.

In these dams, the better milk extraction was under inhalational anaesthesia while for inbred dams, with less weight, the best response was obtained under injectable anaesthesia.

In summary, the milking method described above, provides a valuable means for acquiring substantial amounts of mouse milk. Nevertheless, careful selection of a suitable dam is required, because considerably higher amounts of milk are generally collected from outbred and multiparous in comparison with inbred and primiparous dams.

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